# Methylation Profiling Report

This report has been generated using the **CNS Tumor Methylation Classifier v12.8**. Detailed information about the classifier and description of classes is available at https://app.epignostix.com.

About the **CNS Tumor Methylation Classifier v12.8**:

The classifier was trained based on 7,495 methylation profiles, comprising 184 tumor classes. Utilizing a Random Forest–based methodology, the classifier was rigorously validated through five-fold nested cross-validation, achieving a 95% subclass-level accuracy and a Brier Score of 0.028, indicative of well-calibrated probability estimates. The hierarchical output structure facilitates comprehensive interpretation, allowing clinicians to assess subclass and aggregate class-level probabilities for informed diagnostic decisions. Comparative analyses demonstrate that v12.8 surpasses previous versions and traditional WHO-based diagnostics in prognostic performance across diverse tumor cohorts. Established and distributed under the Molecular Neuropathology (MNP) banner, the CNS Tumor Methylation Classifier has been widely used since 2017.

## Sample Information

| Sample Identifier | Sentrix ID | Array type | Material type | Biological Sex |
|---|---|---|---|---|
| [redacted] | | epic | FFPE | Male |

## Methylation Classifier

| Level | Prediction | Calibrated Score | Interpretation | | Evidence |
|---|---|---|---|---|---|
| Superfamily | Diffuse Glioma, Mapk Altered, Cell-Cycle Activated | 0.9999 | **match** | 3 | |
| Family | Pleomorphic Xanthoastrocytoma(-Like) | 0.9999 | **match** | 3 | |
| Class | Pleomorphic Xanthoastrocytoma(-Like) | 0.9999 | **match** | 3 | |
| Subclass | Pleomorphic Xanthoastrocytoma | 0.9999 | **match** | 3 | A |

**Interpretation Symbol:**
3 **match** (score $\geq$ 0.9)
7 **no match** (score < 0.9)

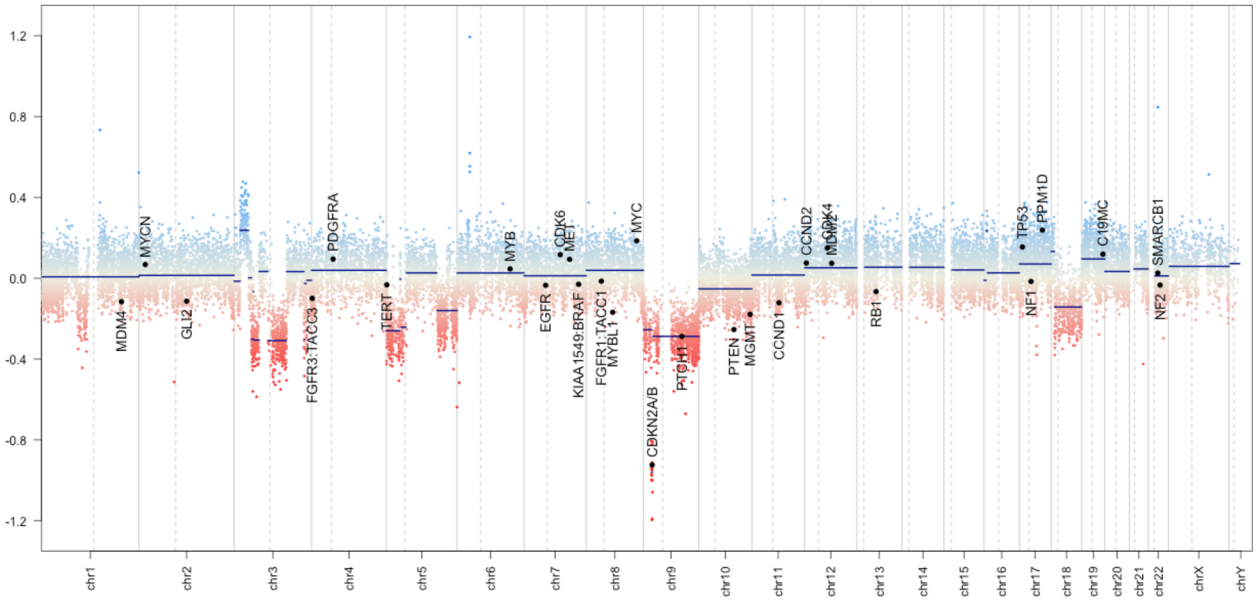
**Evidence Level WHO:**
**Level A** Tumor type/subtype identical to WHO 2021.
**Level B** Large single or more than one smaller dataset published describing the type/subtype as molecularly and/or clinically distinct, or the methylation class represents a distinct fraction of an established WHO 2021 tumor class.
**Level C** Single small dataset or case series.
**Level D** Solely based on clusters in tSNE/UMAP.

## *Description*

The “mc Pleomorphic xanthoastrocytoma” represents an astrocytic tumour with varied histology, primarily that of pleomorphic xanthoastrocytoma or anaplastic pleomorphic xanthoastrocytoma, but also including glioblastomas (particularly epithelioid glioblastoma) or with a ganglion cell-like differentiation appearing as ‘anaplastic ganglioglioma’. There is no

clear difference in methylation profiles between tumours histologically considered anaplastic or not. Location is typically supratentorial and often superficial (involving the leptomeninges). Most case arise in teenagers or young adults, with median age at diagnosis around 20-25 years. There is no apparent sex predilection. The majority of tumours in this mc harbor BRAF V600E mutations and homozygous deletions of CDKN2A/B. Cases lacking BRAF V600E typically show other alterations in the MAPK pathway, including NTRK family and RAF1 gene fusions. Some tumours of this mc also display TERT promoter mutations, which may be associated with a poorer prognosis.

## Copy Number Variation Profile




Generation of the copy number profile of the sample is based on conumee2 (Daenekas et. al 2024). The profile depicts log2 transformed copy number ratios (Y-axis) for chromosomes 1 through 22 (and X/Y if automatic prediction was successful). Potential gains or amplifications are represented by the positive Y-axis, while losses are depicted by the negative Y-axis. 29 CNS tumor relevant gene regions are highlighted.

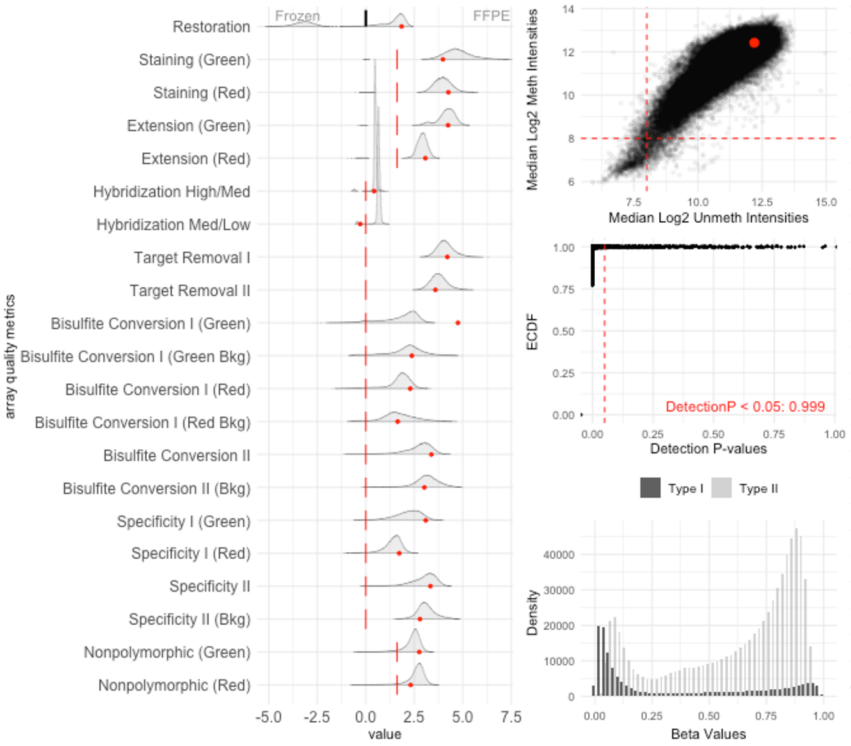
Bjarne Daenekas, Eilís Pérez, Fabio Boniolo, Sabina Stefan, Salvatore Benfatto, Martin Sill, Dominik Sturm, David T W Jones, David Capper, Marc Zapatka, Volker Hovestadt ***Conumee 2.0: enhanced copy-number variation analysis from DNA methylation arrays for humans and mice*** Bioinformatics, Volume 40, Issue 2, February 2024.

## *MGMT* Promoter Methylation (MGMT-STP27)


The MGMT (O-6-Methylguanine-DNA Methyltransferase) gene promoter methylation status is inferred by a logistic regression model developed by Bady et. al 2016. Score of the sample is depicted by the black dot and confidence intervals are shown as whiskers. Red line indicates cutoff for assigning methylation status.

Pierre Bady, Davide Sciuscio, Annie-Claire Diserens et al. ***MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status.*** Acta Neuropathologica, p.547-560, Number 4, 2012.

## Quality Control Metrics




QC Metrics
Array: epic

| | Value | Cutoff | Pass |
|---|---|---|---|
| **Main QC Metrics** | | | |
| Fraction of DetectionP < 0.05 | 0.999 | 0.95 | Pass |
| Median Log2 Meth | 12.420 | 8.00 | Pass |
| Median Log2 Unmeth | 12.196 | 8.00 | Pass |
| **Other QC Metrics** | | | |
| Restoration | 6.327 | 0.00 | Pass |
| Staining (Green) | 53.434 | 5.00 | Pass |
| Staining (Red) | 70.691 | 5.00 | Pass |
| Extension (Green) | 69.554 | 5.00 | Pass |
| Extension (Red) | 21.597 | 5.00 | Pass |
| Hybridization High/Med | 1.536 | 1.00 | Pass |
| Hybridization Med/Low | 0.754 | 1.00 | Fail |
| Target Removal I | 66.914 | 1.00 | Pass |
| Target Removal II | 36.170 | 1.00 | Pass |
| Bisulfite Conversion I (Green) | 115.633 | 1.00 | Pass |
| Bisulfite Conversion I (Green Bkg) | 10.719 | 1.00 | Pass |
| Bisulfite Conversion I (Red) | 9.880 | 1.00 | Pass |
| Bisulfite Conversion I (Red Bkg) | 5.164 | 1.00 | Pass |
| Bisulfite Conversion II | 29.369 | 1.00 | Pass |
| Bisulfite Conversion II (Bkg) | 20.454 | 1.00 | Pass |
| Specificity I (Green) | 21.974 | 1.00 | Pass |
| Specificity I (Red) | 5.592 | 1.00 | Pass |
| Specificity II | 27.979 | 1.00 | Pass |
| Specificity II (Bkg) | 16.292 | 1.00 | Pass |
| Nonpolymorphic (Green) | 15.745 | 5.00 | Pass |
| Nonpolymorphic (Red) | 10.091 | 5.00 | Pass |

On the left, the figure shows 21 quality metrics derived from the control probes on the Illumina Methylation Arrays. Red dots indicate the values for the analyzed sample, while the surrounding distributions reveal the variability observed in our database. Red vertical lines mark the recommended thresholds. Overall array quality is assessed using three primary criteria: (1) the proportion of CpG probes with a detection p-value below 0.05, (2) the log2 median intensity of methylated signal, and (3) the log2 median intensity of unmethylated signal. A sample is considered acceptable for methylation classification when all three of these benchmarks meet or exceed their thresholds. Although slight deviations in other quality metrics may not impact classification accuracy, they might point to issues during laboratory protocols; hence, simultaneous failures in multiple metrics should be investigated further.

## Disclaimer

Classification using methylation profiling is a tool for research use only, it is not verified and has not been clinically validated and, therefore, must not be used for diagnostic purposes. This tool is not HIPAA compliant.

## Run information

Report generated on: 14 May 2025, 14:42:40

**epxCNS package version:** 1.1.0

**CNS Classifier version v12.8**